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Original articles - Newborn

Nicotine affects the expression of brain-derived neurotrophic factor mRNA and protein in the hippocampus of hypoxic newborn piglets

Jannicke Hanne Andresen[1,*], Else Marit Løberg[2], Marianne Wright[1], Ingeborg Løstegaard Goverud[2], Babill Stray-Pedersen[3] and Ola Didrik Saugstad[1]

[1] Department of Pediatric Research, Institute of Surgical Research, Medical Faculty, University of Oslo, Rikshospitalet University Hospital, Oslo, Norway
[2] Department of Pathology, Ullevål University Hospital, Oslo, Norway
[3] Department of Obstetrics and Gynecology, Medical Faculty, University of Oslo, Rikshospitalet University Hospital, Oslo, Norway

Abstract

Brain-derived neurotrophic factor (BDNF) is highly expressed in the developing brain. It has anti-apoptotic abilities, and protects the neonatal brain. In experimental settings in adult animals, pre-treatment with nicotine has shown increased BDNF levels, indicating a possible contribution to nicotine's anti-apoptotic effect. Apoptosis contributes to the development of brain damage in perinatal asphyxia. We examined the effects of nicotine on apoptosis-inducing factor (AIF), caspase-3 and BDNF in the hippocampus of a neonatal piglet model of global hypoxia. Forty-one anesthetized newborn piglets were randomized to one of four groups receiving different infusions after hypoxia (1) nicotine 130 μg/kg/h, 2) 260 μg/kg/h, 3) adrenaline, and 4) saline, all 2.6 mL/kg/h. Four hours after hypoxia they were euthanized. The left hemisphere/hippocampus was examined by histopathology and immunohistochemistry; the right hippocampus was analyzed using real time PCR. There was a significantly higher expression of BDNF mRNA and protein in the animals treated with nicotine 130 μg/kg/h vs. the saline treated group (mRNA $P = 0.038$; protein $P = 0.009$). There were no differences regarding AIF or caspase-3. We conclude that nicotine (130 μg/kg/h), infused over 1 h after global hypoxia in neonatal piglets, increases levels of both BDNF mRNA and protein in the hippocampus. This might imply neuroprotective effects of nicotine in asphyxiated neonates.

Keywords: Apoptosis-inducing factor; brain-derived neurotrophic factor; base excess; hippocampus, nicotine.

*Corresponding author:
Jannicke Hanne Andresen, MD, PhD
National Women's Health
Newborn Services
Auckland City Hospital
Park Road, Grafton
Private Bag 92024
Auckland, New Zealand
Tel./fax: +64 9 307 49 49 extn. 25366/+64 9 375 4344
E-mail: jannickea@adhb.govt.nz

Introduction

Worldwide, perinatal asphyxia has a substantial impact on neonatal morbidity and mortality [4, 22]. Treatment options are scarce, with therapeutic hypothermia being the most promising intervention so far [25]. It has, however, not proven fully capable of preventing the secondary damage seen in perinatal asphyxia, and thus there is still an ongoing search for interventional strategies. Erythropoietin and xenon-gas are examples of strategies that are currently being studied [7, 23]. Another strategy that has been investigated is the use of brain-derived neurotrophic factor (BDNF). BDNF is highly expressed in the developing brain, supporting the survival and maintenance of specific populations of neurons both in the peripheral and central nervous system [19, 24]. It has anti-apoptotic abilities, blocking the activation of caspase-3, and decreasing the up-regulation of other pro-apoptotic proteins (phosphorylated c-Jun, cytochrome C) when administered intracerebroventricular or intraocular in rats [11, 20]. BDNF does not cross the blood brain barrier, and must thus be administered directly intracerebrally [12]. This limits the use in clinical contexts. Agents that increase levels of BDNF could, however, be of use. Nicotine has been shown to have neuroprotective abilities in animal and *in vitro* experiments [13, 28, 30, 31]. This has also been demonstrated in neonatal models [3, 5, 18]. Nicotine acts both as an anti-inflammatory and an anti-apoptotic agent [13, 26], and has been found to increase levels of the anti-apoptotic BDNF [8, 29]. Apoptosis is a major contributor to cell death in perinatal asphyxia [4, 33], and is executed via at least three pathways. One is caspase-independent and involves the apoptosis-inducing factor (AIF). AIF can also induce caspase-activation by triggering the release of mitochondrial cytochrome C. The other two involve the intrinsic pathway (apoptosome formation and caspase-9 cleavage) and the extrinsic pathway (binding of the Fas-ligand to

its receptor and subsequent caspase-8 cleavage), both leading to caspase-3 activation, a key executioner of apoptosis [32, 34, 35].

Nicotine effect on apoptosis and on BDNF has so far not been demonstrated in neonatal hypoxic animal models. We wished to examine the nicotine effects on AIF, caspase-3 and BDNF in a well-established neonatal piglet model of global hypoxia. We hypothesized that nicotine in this setting would decrease the levels of AIF and caspase-3 mRNA expression in the hippocampus; increase the levels of BDNF mRNA-expression in the hippocampus, and increase the levels of BDNF-protein in the hippocampus measured by immunohistochemistry. This would imply a possible neuroprotective effect of nicotine in hypoxic brain damage in the neonate.

Materials and methods

Approval

The Norwegian Council for Animal Research approved the experimental protocol. The animals were cared for and handled in accordance with the European Guidelines for Use of Experimental Animals, by certified FELASA (Federation of European Laboratory Animals Science Associations) category C researchers.

Surgical preparation and anesthesia

Forty-one Noroc (LYxLD) pigs were included in the study, inclusion criteria being age of 12–36 h, hemoglobin values >5 g/dL, and good general condition. Anesthesia was induced by Sevoflurane 5% (Sevorane, Abbott), reduced to 2% before an ear vein was cannulated, Sevoflurane was disconnected, and the piglets were given pentobarbital sodium 20 mg/kg and Fentanyl 50 μg/kg intravenously as bolus injections. Anesthesia was maintained by a continuous infusion of Fentanyl (50 μg/kg/h) and Midazolam (0.25 mg/kg/h; IVAC P2000 infusion pump). When necessary, a bolus of Fentanyl (10 μg/kg) or Midazolam (1 mg/kg) was added. When shivering occurred that did not cease with additional anesthetics, the animals were given pancuronium bromide (0.1 mg/kg). A continuous i.v. infusion (saline 0.7% and glucose 1.25%, 10 mL/kg/h) was given throughout the experiment. The animals were surgically prepared with tracheotomy and insertion of femoral catheters, and ventilated as described in a previous study [3]. Rectal temperature was maintained between 38.5 and 40°C with a heating blanket and a radiant heating lamp. Mean arterial blood pressure (MABP) was measured continuously in the left femoral artery using BioPac systems MP150-CE.

Experimental protocol

All the animals were subjected to global hypoxia after a stabilization period of 90 min. The global hypoxia was induced by 8% O_2 in N_2 until base excess (BE) ≤ -20 mmol/L or MABP = 15 mm Hg. At the end of hypoxia the animals were randomized to one of four groups with different infusions given over 1 h. Animals of both genders were used, and we aimed at a 50/50 distribution in the different groups. The groups were as follows: 1. Nicotine (nicotine hydrogen tartrate, Sigma-Aldrich, Oslo, Norway) 130 μg/kg/h (n = 13); 2. Nicotine 260 μg/kg/h (n = 10); 3. Saline (n = 9); 4. Adrenaline 0.05 μg/kg/min + saline (n = 9). All infusions were given in the dose of 2.6 mL/kg/h and started 5 min after the end of hypoxia. All infusions were given separately in a central venous line. Other drugs and fluids were given through a peripheral line for the duration of the infusion. After the infusions were ended, the animals were observed for 3 h, and were thereafter given an overdose of 150 mg/kg pentobarbital intravenously. The rationale for giving the different groups the mentioned infusions was as follows: nicotine 130 μg/kg/h has been used previously with effect [1], nicotine 260 μg/kg/h was chosen to assess if it was too high to have an effect, or if a higher dose than 130 μg/kg/h would give an even better effect. Adrenaline was given in a dose that mimics the starting dose for hypotensive neonates in the intensive care units; this group was added to imitate nicotine effect on the sympathetic nervous system, to assess the impact of this effect on nicotine neuroprotective abilities. The saline treated group was the control group.

Blood sampling

Hemoglobin was measured on a HemoCue Hb 201 + (HemoCue AB, Angelholm, Sweden) at baseline. Temperature-corrected arterial acid/base status and glucose were measured regularly with a Blood Gas Analyzer 860 (Ciba Corning Diagnostics, Midfield, MA), the blood being obtained from the femoral artery catheter. Two mL of blood for analyses of nicotine concentrations were drawn after the infusion was ended, and frozen at –70°C until analyzed as previously described [2]. All drawn blood was immediately replaced with saline 1.5 times the volume removed.

Tissue sampling

The brain was immediately removed after the overdose had been administered, and the left hemisphere and the cerebellum were placed in 4% buffered formalin at a temperature of 4°C. The posterior part of the right hippocampus was immersed in RNASafer® (Stabilizer reagent, Omega Bio-tek Inc., Norcross GA, USA) and frozen at –70°C for later real-time PCR analyses of BDNF, AIF and caspase-3 gene expression.

Real-time polymerase chain reaction (real-time PCR)

The hippocampus samples were homogenized using MagNA Lyser Green Beads tubes (Roche Diagnostics GmbH, Mannheim, Germany) and lysis buffer. Total RNA from the supernatant was prepared using a Total RNA Kit from E.Z.N.A., and treated with DNase I (E.Z.N.A. Total RNA Kit, Omega Bio-tek). Extracted total RNA was quantified using an ND-1000 spectrophotometer (NanoDrop Technologies, Inc., USA). Total RNA (2–3 μg) was reverse transcribed into cDNA employing the High Capacity cDNA Archive Kit (Applied Biosystems Inc.) in a GeneAmp PCR System 9700 thermal cycle according to the manufacturer's protocol. Real-time PCR was performed with 20 ng cDNA for the target genes and housekeeping gene (PPIA), employing the SYBR Green PCR Master mix in an ABI PRISM® 7000 Sequence Detection System in a universal instrument setting. The following primers and primer concentrations were used: (BDNF: 5′AGCGTGTGCGACAGCATTAG (sense), 5′GTCCACTGCCGTCTTTTTATCC (antisense); AIF: 5′AGGACTCCTTCCATCACAATGTG (sense), 5′TTGGCAAACCCCCTTTCC (antisense); caspase-3: 5′AGCGCTGAAACAGTATGTTCACA (sense), 5′TTCTACTGC-

TACCTTTCGGTTAACC (antisense); PPIA: 5′ATA CGG GTC CTG GCA TCT TG (sense); 5′AAC TGG GAA CCG TTT GTG (antisense).

Real-time PCR quantification of nucleic acids was done by employing the comparative C_T method of relative quantification (RQ). RQ was calculated for each group using ΔCt (normalized to the endogenous control). The arithmetic formula $2^{-\Delta Ct}$ was used for the values displayed in Figure 1.

Pathological examination

Fixation and staining Tissue blocks (0.5 cm thick) from striatum, hippocampus, cortex and cerebellum were embedded in paraffin, sliced in 4 μm thick sections and stained with hematoxylin and eosin (H&E).

Immunohistochemistry Immunohistochemistry with MAP-2 (microtubule-associated protein 2) was performed as previously described [1]. For BDNF the formalin-fixed paraffin-embedded sections were deparaffinized, rehydrated and demasked in a microwave oven for 25 min in TRIS/EDTA buffer at pH 9.1. Monoclonal anti-human BDNF antibody titer 1:25 (Clone 35928; R&D Systems, Minneapolis, MN, USA) was used as primary antibody. This antibody has previously been found to have 100% sequence homology to the porcine antibody [24]. The antigen-antibody reaction was visualized with DakoCytomation EnVision+ System-HRP (K4007; Dako, Carpinteria, CA, USA), using 3.3′-diaminobenzidin as the chromogen. The sections were counterstained with hematoxylin.

Evaluation of histopathology The samples were evaluated by a pathologist who was blinded to the randomization, and only had the numbers of the animals as ID. Areas with vacuolated neuropil, shrunken neurons with pyknotic nuclei and scattered eosinophilic neurons, were defined as necrosis. MAP-2, which is a sensitive marker for neuronal ischemic injury [14], was used to confirm the areas of damage. There was a good correlation between the morphologic changes in the HE-stained sections and loss of MAP-2 staining. Vacuolated areas in subcortical white matter damage were assessed as necrosis, graded as either present or non-present. Necrosis in striatum, hippocampus and cortex was divided into five different categories as shown in Table 1. For the cerebellum, the hypoxic/ischemic changes were defined by the presence of necrotic Purkinje cells with eosinophilic cytoplasm. In each case the number of eosinophilic Purkinje cells was counted in one section from the vermis of the cerebellum. Damage was classified as shown in Table 1.

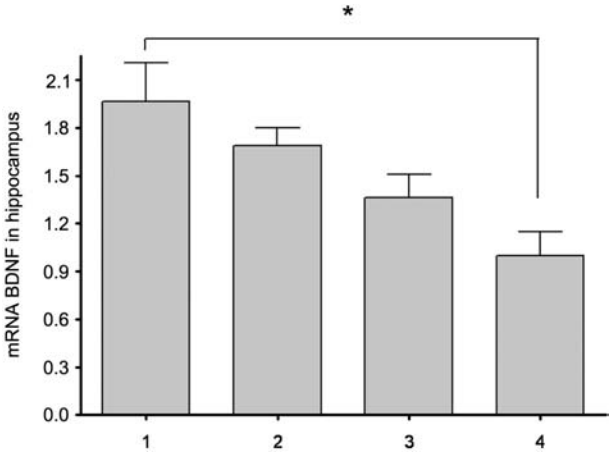

Figure 1 Relative expression of BDNF mRNA in hippocampus. The group treated with nicotine 130 μg/kg/h show a significant increase in BDNF mRNA expression compared to the group treated with saline.
1 = nicotine 130 μg/kg/h; 2 = nicotine 260 μg/kg/h; 3 = adrenaline 0.05 μg/kg/min + saline; 4 = saline. *P = 0.038 for nicotine 130 μg/kg/h vs. saline.

Table 1 Grading of damage in striatum, hippocampus, cortex and cerebellum.

Grade	Degree of damage
Striatum, hippocampus and cortex	
0	No necrosis
1	≤10% of tissue necrotic
2	20–30% of tissue necrotic
3	40–60% of tissue necrotic
4	>75% of tissue necrotic
Cerebellum	
0	No eosinophilic Purkinje cells
1	<50 eosinophilic Purkinje cells
2	50–150 eosinophilic Purkinje cells
3	>150 eosinophilic Purkinje cells

Evaluation of BDNF immunohistochemistry Gyrus dentatus was chosen as the area to evaluate, due to the fact that this is the area of most BDNF-protein according to Kokaia et al. [16]. Positive neurons were identified as staining a dark brown color, while negative neurons had a blue or light brown coloring similar to background staining. We counted positive and negative cells in three areas at 40× enlargement, and calculated the percentage of positive neurons for further analyses (positive cells divided through the total number of cells). We only colored the hippocampi of the animals from the groups treated with 130 μg/kg/h and saline, choosing these two groups since they were the most interesting to compare. The evaluations were done blinded.

Statistical analysis

Statistical analyses were performed by SPSS version 15 (SPSS Inc., Chicago, IL, USA). All values are presented as mean ± SD. Weight, age, Hb, time of endured hypoxia and real-time PCR results were calculated using one-way ANOVA with post hoc tests where appropriate. Immunohistochemistry results were calculated using Student's *t*-test. All analyses for repeated measures were done using multivariate GLM (general linear model) with post hoc tests where appropriate. Histopathological findings were calculated using ordinal regression, for white matter damage binary logistic regression was used. A $P < 0.05$ was considered statistically significant.

Results

Piglets

The mean age of the animals was 27 (±3) h, weight 1970 (±140) g, hemoglobin was 7.5 (±1) g/dL, and the time

the animals endured hypoxia was on average 62 (±24) min, median time being 58 min (range 30–150). There were no significant differences between the groups regarding these parameters. A total of 22 of the 41 animals were male, with the following gender distribution within the groups: nicotine 130 μg/kg/h 7/13 male; nicotine 260 μg/kg/h 5/10 male; saline 5/9 male; adrenaline 5/9 male. There was a significant difference between nicotine 260 μg/kg/h and adrenaline regarding MABP at the end of the infusion (42±10 vs. 53±8 mm Hg; P=0.018) and at the end of the experiment (40±7 vs. 51±13 mm Hg; P=0.043); and there was also a significant difference regarding MABP for nicotine 260 μg/kg/h vs. nicotine 130 μg/kg/h at the end of the experiment (40±7 vs. 54±16 mm Hg; P=0.009). pCO_2, pO_2, BE, pH, temperature and blood glucose showed no significant differences between the groups (Table 2). In all but three of the animals hypoxia was ended due to a decrease in BE (i.e., in 93% of the cases). As shown in Table 2 there were no significant differences between the groups regarding BE and MABP at the end of hypoxia.

Nicotine concentrations The groups treated with saline or adrenaline had nicotine concentrations in full

Table 2 Physiological data.

	Nicotine 130 μg/kg/h n=13	Nicotine 260 μg/kg/h n=10	Saline n=9	Adrenaline n=9
MABP (mm Hg)				
B	66±9	68±14	74±18	73±13
EH	26±8	23±7	24±6	27±7
EI	51±14	42±10*	48±6	53±8*
EE	54±16§	40±7**§	38±9	51±13**
B	2±2.9	3±3.3	1±3.1	3±3.6
BE (mmol/L)				
EH	–20±2.5	–20±1.8	–21±0.6	–20±1.9
EI	–5±4.3	–7±2.3	–7±2.4	–7±1.7
EE	0±4.2	–1±3.5	–5±6.0	–1±1.8
pH				
B	7.44±0.04	7.45±0.05	7.43±0.06	7.45±0.05
EH	7.06±0.05	7.06±0.04	7.05±0.04	7.07±0.05
EI	7.33±0.08	7.30±0.03	7.30±0.04	7.32±0.04
EE	7.40±0.08	7.40±0.05	7.32±0.09	7.39±0.03
pCO_2 (kPa)				
B	5.1±0.2	5.2±0.5	5.2±0.4	5.3±0.6
EH	4.7±0.4	5.0±0.5	4.7±0.9	4.7±0.4
EI	5.2±0.5	5.4±0.3	5.2±0.4	5.0±0.3
EE	5.3±0.4	5.1±0.5	5.7±0.3	5.3±0.3
pO_2 (kPa)				
B	12.5±1.9	12.0±1.5	12.6±1.2	13.3±3.6
EH	4.5±0.3	4.3±0.5	5.0±1.1	4.9±1.0
EI	11.4±1.6	10.7±1.2	11.8±1.3	11.0±0.7
EE	12.1±1.9	11.1±2.1	12.0±1.5	11.3±0.6
Temp (°C)				
B	39.7±0.2	39.6±0.2	39.4±0.2	39.5±0.2
EH	39.2±0.2	39.3±0.2	39.1±0.3	39.3±0.2
EI	39.6±0.3	39.6±0.2	39.7±0.2	39.7±0.2
EE	39.2±0.2	39.2±0.3	39.2±0.3	39.2±0.3
Blood-glucose (g/dL)				
B	6.2±1.0	6.1±1.0	6.7±1.0	6.3±0.7
EH	11.9±9.1	8.7±4.3	10.6±2.1	9.6±3.4
EI	8.2±3.1	7.7±4.0	8.9±3.1	7.8±2.8
EE	6.1±2.3	7.3±2.7	7.7±2.7	6.0±1.1

Physiological data at four different time points throughout the experiment.
*,**indicates significant difference for nicotine 260 μg/kg/h vs. adrenaline (*P=0.018, **P=0.043).
§Indicates significant difference for nicotine 260 μg/kg/h vs. nicotine 130 μg/kg/h (P=0.009).
B, baseline; BE, base excess; EH, end hypoxia; EI, end infusion; EE, end experiment.

blood of 0 ng/mL, the group treated with 130 μg/kg/h: 64±21 ng/mL, and the group treated with 260 μg/kg/h: 135±35 ng/mL ($P<0.001$).

Real-time PCR of BDNF, caspase-3 and AIF

The relative expression of BNDF mRNA showed a significant up-regulation in the hippocampus for the group treated with nicotine 130 μg/kg/h compared to the saline treated group ($P=0.038$, Figure 1). For caspase-3 and AIF, the real-time PCR measurements of the relative expression of mRNA in hippocampus presented no significant differences between the groups. We also did calculations for the two genders separately, but this did not present any significant findings.

BDNF immunohistochemistry

As mentioned, we only assessed the group treated with nicotine 130 μg/kg/h and the group treated with saline. We found a significant difference between the groups, with the group treated with nicotine 130 μg/kg/h presenting 33% (±14) positive cells, and the saline group presenting 17% (±8) ($P=0.009$, Figures 2–4).

Histopathology and MAP-2 in striatum, hippocampus, cortex, cerebellum and subcortical white matter

The damage in the striatum and cortex varied from no damage to 40–60% of the tissue damaged; there were no significant differences between the groups. However, in the striatum there was a tendency towards less damage in the group treated with nicotine 130 μg/kg/h with 2/13 (one <10% and one 20–40% damage) vs. 3/9 (one <10% and two 40–60% damage) animals in the saline group, as illustrated in Figure 5. MAP-2 confirmed the findings. In hippocampus six animals presented damage between 20% and 60%, these were the same animals that presented more severe damage in the cortex and

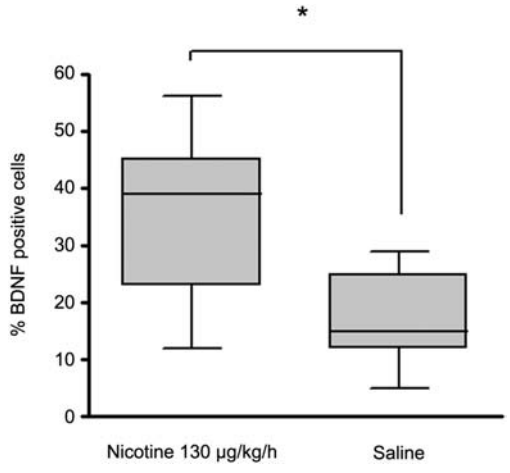

Figure 2 Box plot of % BDNF positive cells in the dentate gyrus of the hippocampus.
There are significantly more BDNF positive cells in the dentate gyrus of the animals treated with nicotine 130 μg/kg/h compared to the saline treated animals. *$P=0.009$.

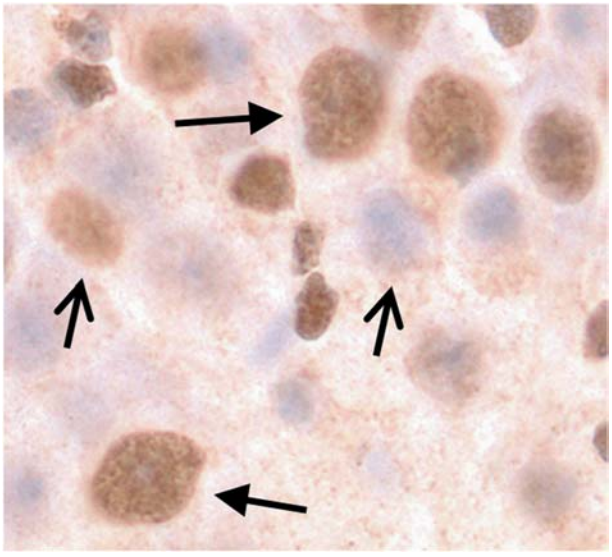

Figure 3 Immunohistochemistry of BDNF protein.
Picture from the dentate gyrus 40×enlarged. The cells with the large, dark brown nucleuses are positive (black arrows), the cells with light brown or blue nucleuses are negative (open arrows).

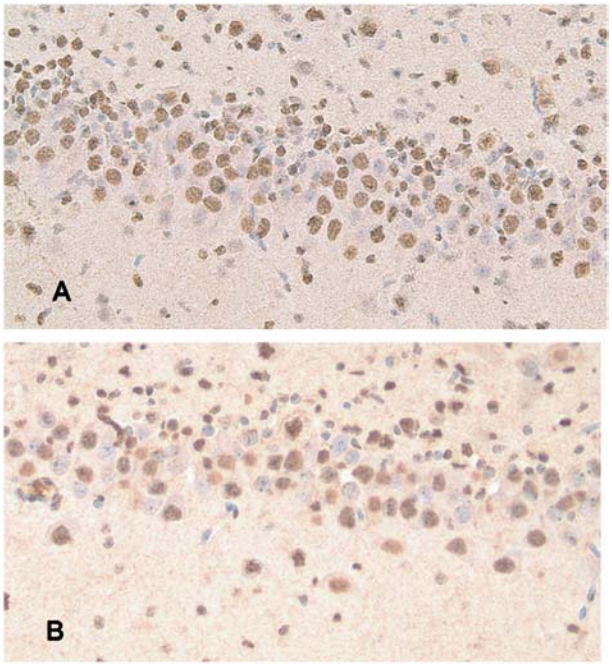

Figure 4 Immunohistochemistry of BDNF protein, nicotine vs. saline.
Pictures from the dentate gyrus 20×enlarged. A is from an animal treated with nicotine 130 μg/kg/h; B is from an animal treated with saline; picture A presents a larger number of BDNF positive cells than picture B.

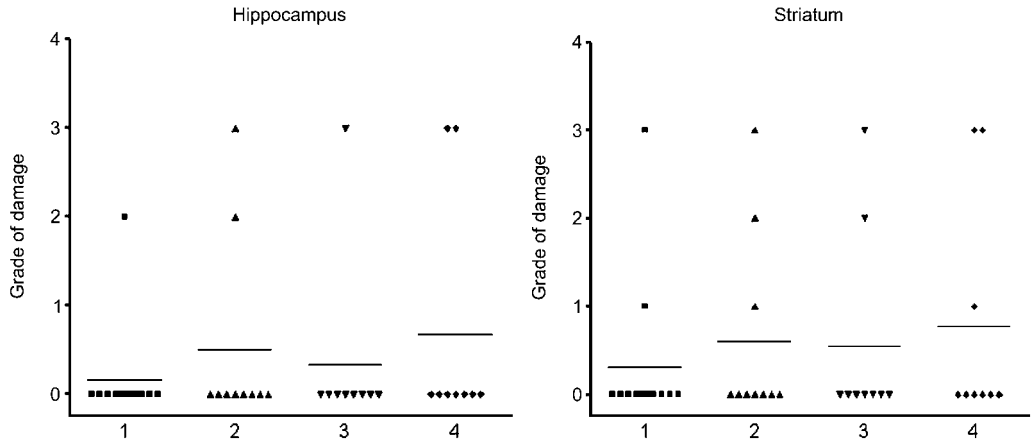

Figure 5 Scatter plot of histopathological damage in hippocampus and striatum.
The trend towards less damage in the group treated with nicotine 130 μg/kg/h is illustrated in these scatter plots. 1 = nicotine 130 μg/kg/h; 2 = nicotine 260 μg/kg/h; 3 = adrenaline 0.05 μg/kg/min + saline; 4 = saline.

striatum as well. The remaining animals had no damage in the hippocampus, and there were no significant differences between the groups. As illustrated in Figure 5 there was, however, a tendency towards less damage in the group treated with nicotine 130 μg/kg/h, with 1/13 (20–30% damage) vs. 2/9 (both 40–60% damage) animals in the saline group. For the cerebellum all the three categories of damage were present (Table 1), but none of the cases with the most severe damage were seen in the animals treated with low dose of nicotine or adrenaline. Subcortical white matter damage was seen in a total of 12 animals. This did not necessarily occur together with necrosis in other areas. There were no significant differences between the groups. There was no difference between the animals presenting white matter damage and the other animals regarding acidosis, MABP, or duration of endured hypoxia.

Discussion

In this study we show that nicotine in a low dose increases BDNF in the hippocampus in a neonatal model of hypoxic brain damage. This finding is in accordance with findings in adult animal models, suggesting neuroprotective effects in the neonatal cohort.

It is unfortunate that the immunohistochemistry was not conducted on all the animals. We do, however, believe our results to be representative even without the two other groups, and the results are in concordance with the findings on mRNA.

We failed to show any effect of nicotine on apoptosis in this study. There were no differences between the groups regarding mRNA expression of AIF or caspase-3. This could be due to a lack of effect of nicotine on both the caspase-dependent and -independent pathways, but it could also be due to the short observation period. Nicotine has been shown to have an effect on apoptosis/caspase-3 *in vitro* [9, 13]. Garrido et al. showed a maximal effect on caspase-3 after 12 h, with no statistical difference between the groups at 3 h [9]. Immunohistochemical staining with caspase-3 and TUNEL could have been beneficial for the evaluation of apoptosis in our study, but this was not done due to the short observation time. It has been shown that there is a distinct difference between genders regarding apoptosis and pathways [27], and gender should, therefore, be taken into account when evaluating apoptosis. Renolleau et al. [27] reviewed experiments in neonatal rodents and cell cultures, presenting evidence that the male population predominantly activates the AIF-dependent pathway, whereas the female population presents a stronger activation of caspase-3. We included both genders in our study, aiming at an equal distribution in the groups. When we subdivided the groups into genders we did not find any significant differences, but the groups were small and thus not statistically reliable. We believe it is not possible to draw any conclusions regarding nicotine effects on apoptosis in our model until more research has been conducted.

Regarding the histopathological findings, there were no statistical significant differences between the groups in any of the investigated regions. We do, however, find a trend towards less injury in striatum and hippocampus in the animals treated with the low dose of nicotine compared to the control group. To properly assess the effect of intervention after hypoxia-ischemia on histopathological outcome, survival studies are necessary. This was not possible with our current model.

We found a significant difference in MABP between the higher dose of nicotine and the lower dose at the end of the experiment, and between the higher dose of nicotine and adrenaline after the infusion and at the end of the experiment. The higher dose of nicotine failed to present

a significant effect on BDNF mRNA, although Figure 1 shows a better effect in this group than in the saline and the adrenaline groups. One could speculate if the lower MABP could have caused the lack of effect on BDNF mRNA. The group treated with adrenaline, however, did not show any beneficial effects of a higher MABP, and it is thus likely that MABP is not important for the effects on BDNF mRNA. Nicotine is known to activate the sympathetic nervous system in a dose-dependent manner, but we have previously demonstrated that the doses used in the current study do not increase plasma catecholamines [2]. The difference in effect between the two nicotine doses can thus not be explained by the difference in MABP – and one can speculate if the higher dose might be too high to have a beneficial effect.

BDNF treatment has been shown to be beneficial in animal models of hypoxia, presenting anti-apoptotic effects [11]. Cheng et al. present an age-dependent neuroprotection with improved effect in rats at postnatal day 7 compared to adult animals [6]. Kokaia et al. find regional differences in the response of BDNF to hypoxia-ischemia in the hippocampus, with gyrus dentatus and CA3 as areas that present high levels of BDNF at baseline and a significant increase after hypoxia-ischemia [16]. Unpublished results from our laboratory show that BDNF increases after hypoxia-ischemia in the current model, and this is in accord with findings of asphyxiated newborns presenting increased levels of BDNF in CSF [17]. Regarding nicotine and neurotrophic factors, French et al. [8] presented a significant increase in the nerve growth factor (NGF), and a trend towards an increase in BDNF after administration of nicotine locally in the hippocampus. They also present an increase in TrkB, the corresponding receptor to BDNF, which is also believed to have antiapoptotic abilities. Garrido et al. [10] found that nicotine pre-treatment before exposure of spinal cord neurons to arachidonic acid protected against decrease in BDNF levels. Kenny et al. [15], however, found a decrease in BDNF in rat hippocampus after an acute administration of nicotine, and an increase after chronic administration. Their study looks at adult animals, and administers nicotine at a relatively high dose of 0.5 mg/kg. They claim that the acute dose has inhibitory effects, and that when given chronically tolerance develops towards the inhibitory effects of nicotine on BDNF. One could speculate if the mentioned inhibitory effect is due to the dose – and that a smaller dose would not have had the same effect.

It could be discussed if the increase in BDNF is a result of increased injury, with BDNF being produced to counteract the damage inflicted. We do, however, see a trend towards less damage in the hippocampus in the animals treated with a low dose of nicotine, supporting the hypothesis that the nicotine induced increase in BDNF has neuroprotective potential. The mechanisms behind nicotine's induction of BDNF release are not fully understood, but Maggio et al. [21] speculate that it might be a combination of nAChR activation and dopamine release. Serres et al. [29] find that the α7 nAChR subunit might be largely responsible for the increased secretion of BDNF that is seen after nicotine administration in a neuroblastoma cell line.

Nicotine as a neuroprotective agent is well-investigated in adult animal models, but it is a relatively new approach in neonatal medicine. Two groups have looked at nicotine in neonatal rodents – finding protective effects [5, 18]. Laudenbach et al. did, however, find differences in the activation of nAChR subtypes, claiming that the effects in neonates is mediated via different mechanisms than in adults [18]. This warrants more research on the mechanisms by which nicotine exerts its neuroprotective effects in neonatal models.

Weaknesses of the model used in this study are for one the age of the animals; at 12–36 h of age the pigs have already to some extent adapted to extra-uterine life, and the lack of hypercapnia during global hypoxia. These weaknesses limit the comparability with the clinical situation, and the results can hence not be directly transferred to the asphyxiated neonate.

Treatment of asphyxiated neonates is currently not optimal. There is a need for better strategies to attenuate the damage inflicted. A neuroprotective treatment should be effective, easily administered, cost-effective, and effective when given after delivery. Nicotine could be a useful strategy, but further research must be conducted on dose-response, and on finding the optimal dose and duration of treatment. We have previously shown a significant effect on cortical non-protein bound iron and striatal glutamate 2 h after hypoxia (1 h after end of infusion of nicotine) [3], and speculate that a longer duration of treatment might be beneficial.

In conclusion, we find that an infusion with nicotine 130 μg/kg/h for 1 h after global hypoxia in neonatal piglets increases levels of both mRNA BDNF, and of BDNF protein in the hippocampus. This might imply neuroprotective effects of nicotine in asphyxiated neonates.

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